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(Commemoration Issue Dedicated to Professor Tetsuya HANAI On the Occasion of His Retirement)

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Dielectric Properties of Erythrocytes Treated with Membrane-Interacting Antibiotics

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The effects of membrane-interacting antibiotics on sheep and human erythrocytes were studied by a dielectric method. The erythrocytes were treated with gramicidin S, polymyxin B, valinomycin, gramicidin D, amphotericin B, nystatin and nonactin. The treatments changed the dielectric relaxation observed on the intact erythrocytes. With regard to the activity to decrease the relaxation frequency f_c , the antibiotics were classified into two groups: (1) Valinomycin, gramicidin D, amphotericin B and nonactin were effective for the reduction in f_c . (2) Gramicidin S, polymyxin B and nystatin had no effect on f_c . According to dielectric theories based on the interfacial polarization, the decrease in f_c corresponds to the reduction in the cytoplasmic conductivity, indicating a loss of ions in the cytoplasm. The present results, therefore, indicated that valinomycin, gramicidin D, amphotericin B and nonactin facilitate the ion diffusion across the erythrocyte membranes.

KEY WORDS: Dielectric analysis/Permittivity/Relaxation frequency/Ionophore/Erythrocyte/Antibiotic

INTRODUCTION

In a previous paper¹⁾, we confirmed the applicability of dielectric mixture equations in dielectric analysis using erythrocyte suspensions. The purpose of this paper is to apply the dielectric analysis to evaluation of the interaction between erythrocyte membranes and some antibiotics known as an ionophore.

Dielectric measurements were carried out for suspensions of the sheep and human erythrocytes treated with some antibiotics. The data of dielectric measurements were analyzed by using the dielectric mixture equation.

List of symbols

- ϵ : relative permittivity
- κ : electric conductivity
- f_c : characteristic frequency of dielectric relaxation
- C_m : specific membrane capacitance
- Φ : volume fraction of erythrocytes in suspension

Subscripts

- a : external phase
- i : internal phase of erythrocyte
- m : membrane phase

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l : limiting value at low frequencies

h : limiting value at high frequencies

MATERIALS AND METHODS

Preparation of sheep and human erythrocytes

Erythrocytes were obtained from sheep blood (Kohjin Co., Ltd., Tokyo) and human venous blood by centrifugation at $500 \times g$ for 10 min. After removal of the serum and buffy coat, the sediment was washed 3 times with isotonic 10 mM NaCl-glucose solution (285 mOsm/kg H_2O) or phosphate buffered saline. The erythrocytes were resuspended in slightly hypotonic 10 mM NaCl-glucose solution (230 mOsm/kg H_2O), hereafter called "NaCl-glucose solution", which makes the erythrocytes nearly spherical.

Treatment of erythrocytes with membrane-interacting antibiotics

The membrane-interacting antibiotics used were gramicidin S hydrochloride, polymyxin B sulfate, valinomycin, gramicidin D, amphotericin B, nystatin and nonactin, which were all obtained from Sigma (St. Louis, MO, U. S. A.). Gramicidin S hydrochloride and polymyxin B sulfate were dissolved in the NaCl-glucose solution. Valinomycin, gramicidin D, amphotericin B, nystatin and nonactin were dissolved in ethanol and then these ethanol solutions were diluted with five parts of the NaCl-glucose solution.

The erythrocytes were treated by mixing 4.5 ml of one of the antibiotic solutions and 0.5 ml of a concentrated sheep or human erythrocyte suspension. After 30 min treatment at room temperature except the valinomycin treatment (immediately after mixing for the valinomycin treatment) the suspending medium was removed by centrifugation and then the NaCl-glucose solution was added to the sediment so that the final volume is 2 ml. The resulting suspension was subjected to dielectric measurements.

Dielectric measurement

Equivalent parallel capacitance and conductance of the erythrocyte suspensions were measured over a frequency range 10 kHz to 100 MHz by a computer-controlled impedance analyzer system. The system and the measuring cell were exactly the same as those described in a previous paper¹⁾. The measured data were corrected for the residual inductance arising from the measuring cell and its fixture by Schwan's method²⁾ and were also corrected for the electrode polarization by the frequency variation technique³⁾.

Analysis of dielectric data

The dielectric relaxation observed with intact and treated erythrocytes was analyzed by using Pauly-Schwan's theory⁴⁾ based on a single-shell model, that is, a sphere (in this case, cytoplasm) covered with a shell (plasma membrane). The membrane capacitance and the conductivity and permittivity of the cytoplasm were esti-

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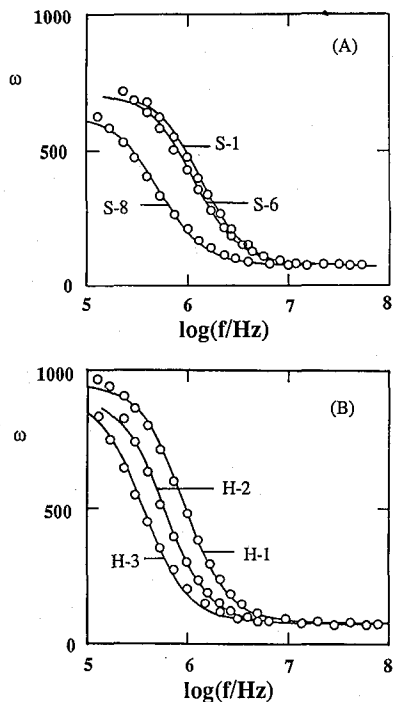


Fig. 1. Plots of relative permittivity(ϵ) of erythrocyte suspensions of sheep(A) and human(B) against frequency(f). The curves in (A) and (B), respectively, refer to the data in Table 1 and 3. The solid curves were calculated from the best-fit phase parameters shown in Table 2 and 4.

mated as phase parameters of the model.

RESULTS AND DISCUSSION

Intact sheep and human erythrocyte suspensions showed a dielectric relaxation with a characteristic frequency of about 1 MHz (Curves S-1 and H-1 in Fig. 1). The dielectric relaxation was affected by the treatments of membrane-interacting antibiotics; typical changes of the dielectric relaxation caused by valinomycin (Curve S-8) and gramicidin D (Curve H-3) are shown in Fig. 1.

Table 1 shows the dielectric parameters obtained with the suspensions of sheep erythrocytes treated with gramicidin S, polymyxin B and valinomycin. The treatments of these antibiotics slightly decreased the limiting value of relative permittivity at low frequencies ϵ_i . The value of the characteristic frequency f_c was almost unchanged by the treatments of gramicidin S and polymyxin B, whereas valinomycin markedly decreased the value of f_c . Table 2 shows the phase parameters obtained by fitting Pauly-Schwan's theory to the data listed in Table 1. These antibiotics all decreased the volume fraction Φ of erythrocytes in suspension, whereas the membrane capacitance C_m was almost unchanged. Valinomycin decreased markedly the conductivity κ_i of the cell interior, suggesting that this antibiotic promote the ion-transfer across the erythrocyte membranes. Although gramicidin S and polymyxin B are known to increase the

Table 1 The dielectric parameters observed for the sheep erythrocytes treated with some antibiotics. The suspending medium is a NaCl-glucose solution.

Specimen	drug	drug concn. ($\mu\text{g/ml}$)	ethanol concn.(%)	ϵ_l	ϵ_h	κ_l (mScm^{-1})	f_c (MHz)
S- 1	none	0	0	710	78	0.747	1.38
S- 2	gramicidin S	153	0	680	78	0.788	1.20
S- 3	gramicidin S	204	0	620	78	0.791	1.30
S- 4	polymyxin B	135	0	640	78	0.818	1.25
S- 5	polymyxin B	180	0	640	78	0.804	1.25
S- 6	none	0	15	710	78	0.772	1.18
S- 7	valinomycin	8.3	15	650	78	0.720	0.53
S- 8	valinomycin	16.7	15	630	78	0.822	0.53
S- 9	valinomycin	24.9	15	600	78	0.818	0.55
S-10	valinomycin	33.2	15	600	78	0.817	0.55

Table 2 The phase parameters calculated from the dielectric parameters in Table 1 by using Pauly-Shwan's equation.

Specimen	ϵ_a	κ_a (mScm^{-1})	Φ	C_m (μFcm^{-2})	ϵ_l	κ_l (mScm^{-1})
S- 1	79	1.054	0.22	1.08	38	2.37
S- 2	78	1.044	0.18	1.20	47	2.33
S- 3	78	1.045	0.18	1.09	47	2.29
S- 4	78	1.050	0.16	1.23	20	2.72
S- 5	79	1.054	0.17	1.16	39	2.38
S- 6	77	1.046	0.19	1.18	62	2.19
S- 7	79	1.061	0.16	1.22	75	0.72
S- 8	78	1.051	0.16	1.22	79	0.72
S- 9	78	1.053	0.16	1.14	79	0.70
S-10	77	1.049	0.16	1.15	84	0.70

Table 3 The dielectric parameters observed for the human erythrocytes treated with some antibiotics. The suspending medium is a NaCl-glucose solution.

Specimen	drug	drug concn. ($\mu\text{g/ml}$)	ethanol concn.(%)	ϵ_l	ϵ_h	κ_l (mScm^{-1})	f_c (MHz)
H-1	none	0	0	950	78	0.877	0.93
H-2	none	0	15	900	78	0.848	0.60
H-3	gramicidin D	17.3	15	900	78	0.841	0.37
H-4	amphotericin B	13.9	15	900	78	0.834	0.45
H-5	nystatin	13.9	15	900	78	0.817	0.63
H-6	nonactin	11.1	15	900	78	0.849	0.41

permeability of *E. coli*. membranes to K^+ ions^{5,6}), the antibiotics did not change the value of κ_l .

Table 3 summarizes the dielectric parameters observed with the human erythrocytes treated with gramicidin D, amphotericin B, nystatin and nonactin. Table 4 shows the estimated dielectric parameters of the erythrocytes. Human erythrocytes

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Table 4 The phase parameters calculated from the dielectric parameters in Table 3 by using Pauly-Schwan's equation.

Specimen	ϵ_a	κ_a (mScm ⁻¹)	Φ	C_m (μ Fcm ⁻²)	ϵ_i	κ_i (mScm ⁻¹)
H-1	80	1.282	0.24	0.62	73	1.60
H-2	79	1.195	0.21	0.63	77	0.94
H-3	80	1.118	0.18	0.72	70	0.63
H-4	78	1.129	0.19	0.69	76	0.76
H-5	78	1.180	0.23	0.60	79	0.94
H-6	80	1.139	0.19	0.71	71	0.69

were more sensitive to ethanol in the treatment solutions than sheep erythrocytes with regard to the dielectric parameters. All of the antibiotics except nystatin decreased the values of f_c and κ_i . This suggests that gramicidin D, amphotericin B, nonactin increases the permeability of the erythrocyte membranes to ions. It is not understood yet why the decrease in κ_i was not observed for the erythrocytes treated with nystatin, which forms channels in lipid bilayer membranes like amphotericin B.

In conclusion, we confirmed that the dielectric analysis is useful for studying the interaction between biological membranes and antibiotics and for evaluating ionophore activity of antibiotics.

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